Anaerobic ammonium oxidation of ammonium-rich waste streams in fixed-bed reactors

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Abstract The feasibility of anaerobic ammonium oxidation (Anammox) in fixed-bed reactors was evaluated on laboratory and pilot scales. Using synthetic wastewater, the specific nitrogen removal rate was increased from 0.05–0.1 kgNm⁻³_reactor d⁻¹ to 0.35–0.38 kgNm⁻³_reactor d⁻¹ within a year (T = 22–27°C) in all applications. However, the anammox activity was seriously and repeatedly inhibited at prolonged high nitrite concentrations (e.g. six days at 30–50 gNO₂-Nm⁻³) and recovery was always a lengthy process. But even at a moderate nitrite concentration (11 ± 10 gNO₂-Nm⁻³), the observed specific growth rate was only 0.018 d⁻¹ at 26.4 ± 0.8°C, which corresponds to approximately 0.025 d⁻¹ at 30°C (doubling time: 28 days). In a second experimental period for another 250 days, one of the laboratory reactors was fed with partially nitrified sludge liquors from a domestic wastewater treatment plant (WWTP). In this case, the specific elimination rate was as high as 3.5 kgNm⁻³_reactor d⁻¹ at 26–27°C. Independently of the feed, the average nitrogen elimination rate lay between 80–85% in all applications. An appropriate hydraulic design is essential to prevent clogging and local nitrite inhibition in fixed-bed reactors.

Keywords Ammonium-rich wastewater; anammox; fixed-bed reactor; nitrogen elimination

Introduction Sustainable nitrogen elimination from ammonium-rich wastewater with a low biodegradable COD content seems to be feasible via anaerobic ammonium oxidation – a novel process in which ammonium is converted to nitrogen gas with nitrite as the electron acceptor (van de Graaf et al., 1996). Given long-term stable operation, this anammox process offers remarkable advantages. The required oxygen demand for nitrification is reduced, no organic carbon source is needed and relatively low excess sludge production with marginal disposal costs can consequently be expected (Strous et al., 1997). The reported maximum specific growth rate for the anammox bacteria is only 0.065 d⁻¹ (Strous et al., 1998), corresponding to a doubling time of 11 days. Hence efficient sludge retention, which can be successfully achieved in biofilm applications, is essential. In this report, we focused on the potential of anaerobic ammonium oxidation in fixed-bed reactors fed with a synthetic medium or partially nitrified sludge-digester effluents. Special attention was given to the start-up period and long-term operation to ensure stable nitrogen elimination at high specific rates.

Materials and methods

Laboratory and pilot plants

Two laboratory fixed-bed reactors (hereafter called FBR 1 and FBR 2) and a pilot plant (FBR 3) were used in these experiments. All non-transparent reactors were inoculated with anammox bacteria containing sludge which was scraped off from a rotating biological contactor treating leachate from a landfill in Köllikon (Switzerland, Egli et al., 2003). The reactor sizes, carrier material and specific surfaces are presented in Table 1. All three reactors were intermittently sparged with N₂ for anaerobiosis (between 15–240 seconds every 2–4
hours). Mixing was provided by an external recirculation pump. For the first 1–3 months of operation, the reactors were operated manually in batch mode, and ammonium, nitrite and minerals were added after depletion. Thereafter, a synthetic influent was continuously taken at room temperature from non-stirred storage tanks which were also sparged with N₂ for the laboratory reactors. The tubes and plugs were made of butyl and nitrile rubber. In a second phase, FBR 2 was supplied with oxygen containing effluent from a nitritation reactor (Fux et al., 2002), but some sludge supernatant was directly added to prevent nitrite accumulation. In all experiments, the pH was kept constant at a pre-set value by the addition of 0.1–1 M HCl.

Influent medium
The synthetic influent comprised 0.19–1.4 g NH₄Cl, 0.25–2.0 g NaNO₂, 17.5 mg Na₂HPO₄·2H₂O, 13.9 mg KH₂PO₄ and 0.1 mL trace element solution 1 (Fux et al., 2004) per litre tap water. For FBR 3, the phosphorus content in the influent was increased to 240 mg Na₂HPO₄·2H₂O per litre after 65 days of operation. The tap water contained 5 mM alkali and 3 g NO₃-N m⁻³ on average. In addition, the influent to the laboratory reactors (FBR 1 and 2) also included 3 g m⁻³ of a yeast product (1 g COD g⁻¹ yeast). All chemicals used were of analytical grade (FLUKA or MERCK).

The sludge liquors were obtained from the sludge dewatering facilities of the Werdhölzli wastewater treatment plant (WWTP) in Zurich. The composition of the supernatant was similar to that described in Fux et al. (2002). The ammonium concentration ranged from 500–750 g NH₄-N m⁻³, about 50–60% of it being nitrified to nitrite. No nutrients were added.

Analytical procedures
Analytical procedures for ammonium, nitrite, nitrate and alkalinity were performed as described in Fux et al. (2002). The phosphate concentration was measured colorimetrically by means of flow injection analysis (FIA).

Calculations
All calculated specific nitrogen elimination rates (kg N m⁻³ reactor d⁻¹) are based on the fixed-bed volume and not on the hydraulic size of the reactor. The observed anammox growth rate is based on exponential fits to the nitrogen turnover rates according to Eq. (1):

\[ r_{Ni,t} = r_{Ni,0} \cdot e^{\mu_i \cdot t} \]  

where

- \( r_{Ni,t} \): Elimination (NH₄-N, NO₂-N, N_total) or production rate (NO₃-N) at a defined time \( t \).

<table>
<thead>
<tr>
<th>Reactor</th>
<th>FBR 1</th>
<th>FBR 2</th>
<th>FBR 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydraulic volume [L]</td>
<td>3.5</td>
<td>3.5</td>
<td>120</td>
</tr>
<tr>
<td>Fixed bed volume [L]</td>
<td>2.54</td>
<td>2.54</td>
<td>70</td>
</tr>
<tr>
<td>Carrier material</td>
<td>PVC¹)</td>
<td>PVC¹)</td>
<td>PP²)</td>
</tr>
<tr>
<td>Specific surface [m² m⁻³]</td>
<td>250</td>
<td>250</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 1 Laboratory and pilot-plant reactor. All reactors were continuously fed. The carrier material was obtained from VA TECH WABAG (Switzerland). PP = polypropylene, PVC = polyvinyl chloride, syn = synthetic medium, liq = sludge liquors.

Generally used for ¹)trickling filters or ²)fixed-bed reactors.
Results

Nitrogen elimination with synthetic feed

All fixed-bed reactors were run up to three months in semi-batch mode for biomass attachment of the inoculum. Thereafter, continuous feeding was installed. In the course of the experiment, the nitrogen load was enhanced either by increasing the inflow or the ammonium and nitrite concentrations. The improvement of the nitrogen removal activity was much slower than expected and the elimination rates were rather modest even after a year of operation. No more than 0.35–0.38 kgN\text{Nm}^{-3}\text{reactor}^{-1}d^{-1} was removed in all three reactors (Table 2). The temperature ranged from 22°C to 27°C.

The overall stoichiometrical ratio of ammonium and nitrite consumption and nitrate production was 1:(1.32 ± 0.08):(0.19 ± 0.01), which is rather similar to the 1:(1.31 ± 0.06):(0.22 ± 0.02) reported by van de Graaf et al. (1996). In order to maintain an optimal pH of around 8.0 (Strous et al., 1997; Egli et al., 2001), hydrochloric acid was dosed. In FBR 3 the acid consumption was measured for 130 days of operation, 0.12 ± 0.01 molHCl being consumed per mole of ammonium removed (average pH = 7.7). This is in accordance with Strous et al. (1998), who reported an overall anammox equation with a proton consumption of 0.13 molH^{+}mol^{-1}NH_{4}^{+}N_{\text{removed}} to keep the pH constant. In several preliminary experiments without acid addition, the pH increased to 9.3, resulting in complete loss of activity.

The low increase of the nitrogen elimination rate is mostly due to severe and irreversible nitrite inhibition as shown in Figure 1 for FBR 1 and FBR 2, and similar results were also obtained for FBR 3 (not shown). In FBR 1, recurrent nitrite concentrations above 50 gNO_{2}Nm^{-3} caused a rather modest performance during the first eight months of operation. The negative influence of high nitrite concentrations was even more obvious in FBR 2. After 130 days of operation, about 80% of the anammox activity was lost at 80 gNO_{2}-N\text{Nm}^{-3}. In the course of the experiment, the nitrogen removal rate could only be re-established after a significant decrease of the nitrite concentration. Similar effects were observed again after 200 and 300 days of operation. However, it is rather difficult to conclude from these experiments whether the absolute nitrite concentration, the exposure time or a combination of both was responsible for the inhibitory effects.

Table 2 Anaerobic ammonium oxidation in fixed-bed reactors. Only the periods fed with synthetic influent are presented. The ammonium effluent concentration always averaged 20–40 gNH_{4}-N\text{Nm}^{-3}

<table>
<thead>
<tr>
<th>Reactor</th>
<th>FBR 1</th>
<th>FBR 2</th>
<th>FBR 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>Days</td>
<td>383</td>
<td>331</td>
</tr>
<tr>
<td>Influent concentration</td>
<td>gNH_{4}-Nm^{-3}</td>
<td>50–220</td>
<td>50–190</td>
</tr>
<tr>
<td></td>
<td>gNO_{2}-Nm^{-3}</td>
<td>50–260</td>
<td>50–240</td>
</tr>
<tr>
<td>Influent load</td>
<td>gNm^{-3}reactor^{-1}d^{-1}</td>
<td>80–420</td>
<td>70–550</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>27 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>pH (controlled)</td>
<td>–</td>
<td>7.3–7.9</td>
<td>7.3–7.9</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>gPm^{-3}</td>
<td>3.6 ± 1.0</td>
<td>3.6 ± 1.2</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>mM</td>
<td>3.3 ± 1.1</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>Nitrite in effluent</td>
<td>gNO_{2}-Nm^{-3}</td>
<td>21 ± 21</td>
<td>18 ± 17</td>
</tr>
<tr>
<td>Initial elimination rate</td>
<td>gNm^{-3}reactor^{-1}d^{-1}</td>
<td>50–100</td>
<td>50–100</td>
</tr>
<tr>
<td>Maximum elimination rate</td>
<td>gNm^{-3}reactor^{-1}d^{-1}</td>
<td>350</td>
<td>380</td>
</tr>
<tr>
<td>Nitrogen turnover [NH_{4}^{+}N degradation: NO_{2}^{-}N degradation:NO_{3}^{-}N production]</td>
<td>1:1.138 ± 0.15: 1:1.34 ± 0.12: 1:1.23 ± 0.05:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NO_{2}^{-}N degradation:NO_{3}^{-}N production</td>
<td>0.19 ± 0.06</td>
<td>0.18 ± 0.05</td>
</tr>
</tbody>
</table>
For no apparent reason, the nitrogen removal rate never exceeded 0.4 kgNm$^{-3}$ reactor$^{-1}$ d$^{-1}$. Phosphate limitation was excluded because no better performance was achieved in FBR 3 even at around 40 gPm$^{-3}$. It is evident that not all the bacteria in the fixed bed experience the same environmental conditions. Depending on the hydraulics, the thickness and density of the biofilm and the different biological reactions involved, significant substrate gradients occur within the biofilm. Due to the anammox process, the pH might rise to sub-optimal levels in the deeper layers. The pH in the bulk of FBR 1 and FBR 2 consequently decreased to 7.3 after 229 and 302 days respectively. However, the subsequent increase of the anammox activity was due to the low nitrite concentration rather than to the pH change. In both cases, the increase in nitrogen elimination was very similar to that recorded in earlier periods of the experiments at low nitrite concentration (e.g. $t = 20–70$ days in FBR 2). Thus the long-term effects of a lower pH seemed to be of minor importance. Because the limiting performance could be due to the synthetic influent medium (missing trace elements or growth factors), the feed to FBR 2 was replaced by partially nitrified sludge liquors for another 250 days while the other two reactors were stopped.

**Using effluent from partial nitritation as influent to FBR 2**

Approximately 100 days passed without any improvement in nitrogen elimination performance (Figure 2). The inflow was switched off at times, whenever no partially nitrified water was available, but we also had to reduce the flow repeatedly to get rid of the remaining nitrite. Thereafter the nitrogen input could be increased continuously.

After 120 days of operation, the nitrogen removal rate was in the range of 0.5–1.5 kgNm$^{-3}$ reactor$^{-1}$ d$^{-1}$ for more than two months before a breakdown of the raw supernatant pump led to nitrite accumulation. The duration of the high nitrite-containing period cannot be determined precisely, but was about 2–3 days. The maximum concentration did
not exceed 50 gNO$_2$-N m$^{-3}$. Fortunately, the anammox bacteria were not seriously inhibited because the original activity was re-established within days. The influent was increased for the last five weeks of operation, resulting in a final nitrogen elimination rate of approximately 3.5 kgNm$^{-3}$ reactor$^{-1}$ at 26–27°C. The overall nitrogen elimination performance was 80% because the separate supernatant addition was not optimized. The ammonium effluent concentration was thus 90 gNH$_4$-N m$^{-3}$ on average, with peaks up to 200 gNH$_4$-N m$^{-3}$.

The amount of the acid addition required depends on the selected pH in the reactor. In this experiment, the pH was controlled at 7.3, resulting in a rather high consumption of 0.21 mole HCl per mole ammonium removed. This is significantly more than the 0.12 molHCl mol$^{-1}$NH$_4$-N described above to maintain the pH at 7.7.

**Growth rate of the anammox bacteria**

Anaerobic ammonium oxidizers grow about ten times more slowly than nitrifiers under optimum conditions. Strous *et al.* (1998) reported a maximum specific growth rate for anammox bacteria of 0.065 d$^{-1}$, corresponding to a doubling time of 11 days. However, the growth rates observed in our experiments were even lower. Figure 3 presents the calculated rates for nitrite and ammonium elimination and nitrate production in FBR 1 for a period of 100 days at a low nitrite concentration (11 ± 10 gNO$_2$-N m$^{-3}$). The total nitrogen elimination rates comprise ammonium and nitrite elimination minus nitrate production and are also depicted in Figure 1. The dashed lines are based on exponential fits for the calculated growth rates according to Eq. (1). The average temperature was 26.4 ± 0.8°C.

The observed growth rate was only 0.018 d$^{-1}$ for all inorganic nitrogen compounds at 26.4°C. Assuming an exponential influence of the temperature on the kinetics with a 10% increase per degree centigrade, the growth rate amounted to 0.025 d$^{-1}$ at 30°C (Figure 3, thin solid line). The corresponding doubling time is thus 28 days, which is 2–3 times longer than reported in Strous *et al.* (1998). The wash-out of the anammox bacteria from the fixed film was assumed to be negligible, but it could also have made some contribution to the low observed growth rate. On the other hand, our anammox bacteria, which are similar but not identical to the reported archetype (Egli *et al.*, 2001), could indeed have a lower growth rate. It was rather difficult to run an anammox fixed-bed reactor without nitrite limitation and inhibition throughout the whole biofilm. The expected doubling time for full-scale operations will therefore probably be closer to a month than to 10 days.

**Conclusions**

Anaerobic ammonium oxidation in fixed-bed reactors is feasible. High specific nitrogen elimination rates were achieved (3.5 kgNm$^{-3}$ reactor$^{-1}$d$^{-1}$), but the start-up period took more than a year. High nitrite concentrations over a prolonged period certainly have a detrimental effect on anammox bacteria. However, further experiments should be performed to...
evaluate the critical concentration and/or exposure time. Although an external recycle pump was installed for vigorous mixing, many sectors of the fixed bed were clogged with solids when the reactors were dismantled. Nevertheless, a suitable hydraulic design is essential to prevent local nitrite inhibition (e.g. close to the dosage area) or anaerobic zones with toxic sulphate reduction (van Dongen et al., 2001). Good mixing and high nitrogen elimination rates can be achieved more easily in sequencing batch reactors with suspended (Fux et al., 2002) or granulated biomass (Strous et al., 1998).

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References